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Taccalonolides are structurally unusual steroid derivatives with microtubule-stabilizing, i.e. Tasol-like activity. The aim of the work is definition of a minimal structural motif that on theone had simplifies the structure but improves of at least retains potency as an antimitotic.
Taccalonolide E, a minor compound found in Tacca chantrieri, has been found to be a more potent cytotoxin that taccalonolide A. The two compounds differ by additional oxygenation in ring C of taccalonolide A. we therefore focused on removing the two acetoxy functions in this ring of taccalonolide A by means of biocatalysis. This approach has so far not proven productive. The use of enzymes immobilized on a suitable solid support is now being pursued with the expectation that improved recovery will be observed.
Functional group removals at other sites in taccalonolide A by chemical means are being pursued.

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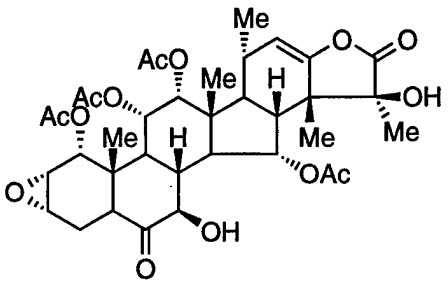
INTRODUCTION

Under prior funding we had discovered the micotubule-stabilizing activity of an unusual, steroid-like molecule from the Asian medicinal plant *Tacca chantrieri.* The results of the biological evaluation and characterization of this material was published earlier this year by our former collaborators.[1] Interestingly, in the intervening years since our original discovery it has been found that a minor taccalonolide, taccalonolide E (**2**), has higher activity in some cell lines than taccalonolide A (**1**), the major compound we had originally isolated. The structural difference between this compound and the major steroid is the level of oxygenation in the C-ring (as indicated by the arrow).



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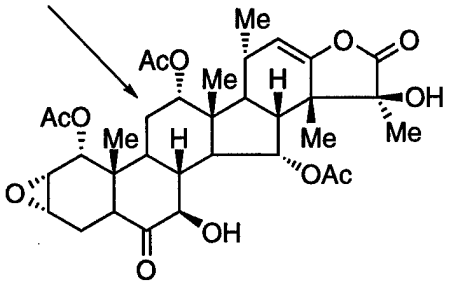

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The aim of the work under the present proposal is the definition of the basic pharmacophore of taccalonolide. The complexity of the natural product does not make it amenable to preparative organic synthesis nor does the potency of the material warrant such an effort at present. Modification of the natural product aimed at deleting some or all of the functionality adorning the periphery of the compound should guide us toward a simpler molecule that is amenable to chemical synthesis. A second goal is the discovery of a more potent derivative since the potency the natural products with an IC_{50} of around micromolar is not particularly high in comparison to other natural products with this biological effect.

BODY

Task 1

Prompted by the report of our former collaborators, we carefully analyzed fractions left over from previous taccalonolide isolation work. We have been able to isolate about 17 mg of taccalonolide E from these collections. In the coming year, we intend to apply to this material some of the chemistry we have already worked out on taccalonolide A. We want to establish whether through further functional group deletions additional increases in potency of **2** are possible.

An additional 45 mg of taccalonolide A was obtained from a new collection of rhizomes for use in our ongoing work on the modification of this compound.

Task 5

The discovery that lower levels of oxygenation in ring C lead to increases in biological potency prompted us to investigate whether a selective removal of the acetate groups in ring C is possible by means of biocatalysis. The need for use of such a technique arises because base catalyzed hydrolysis of the acetate esters is not promising owing to the presence of the enol ester in the E-ring, which is the most easily hydrolyzed ester in the molecule. The rates of the enzyme-catalyzed reactions on the other hand are not primarily determined by the lability of the ester.

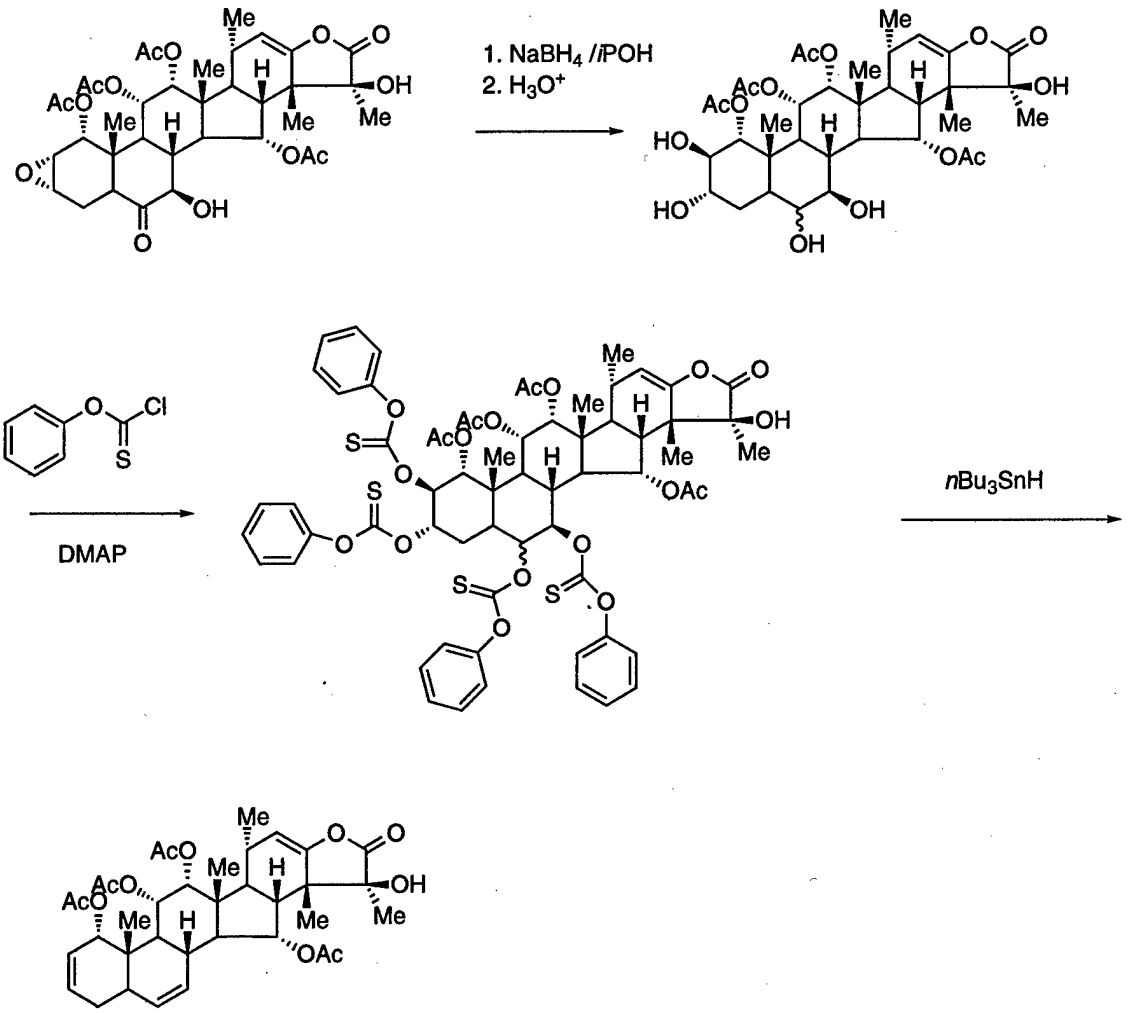
The original work plan had envisaged that we would be able to accomplish the removal of individual acetate residues by using the cross-linked enzyme crystals (CLEC) produced by Altus Biologics. A convenient and relatively affordable screening kit containing samples of all of the esterases marketed by this firm in the form of CLEC's was available at the time. Unfortunately, the company has decided to no longer offer these kits to customers desiring to do their own screening and to provide instead in-house screening as a service with substrates supplied by the customer. The price tag of such services puts it out of reach of an academic investigator, however, and we had to abandon the use of CLEC's.

We have attempted instead to use commercially available esterases with taccalonolide A as a substrate in order to achieve selective deacylation. All attempts have so far failed as the student working on this problem could not solve a variety of technical problems in separating the substrate/product from the enzyme and isolating the taccalonolide or the product derived therefrom. The recovery of material was uniformly bad on the necessarily small scale. To avoid further unproductive loss of scarce starting material, the approach was abandoned for the time being. It is hard to imagine that the problems should be insurmountable in the right hands. Alternatively, immobilized enzymes on solid supports may be used. Lipases immobilized on Sol-Gel-AK have become commercially available from Fluka/Aldrich this year. This should allow, as we had envisioned for the CLEC's, the selection of a physical method for the separation of the biocatalyst from the substrate and the products. The use of polar supports such as Sol-gel is crucial for the success of the reaction because the beads employed in classical enzyme immobilization will adsorb the substrate and products.

We have continued also our studies on the purely chemical modification of taccalonolide A. Task 5 envisaged the deletion of multiple functional groups within a derivative. This requires the execution of several multi-step procedures on one compound and, unsurprisingly, this is hard to do on small scale. Several of the procedures reported in prior reports for this project on the deletion of single functional groups have chemical yields in the 60% range. It is therefore to be expected that the combination of two such procedures in sequence for the deletion of two functional groups will return overall yields

of 30% and often lower after purification. We may have bitten off more than we can chew on this one. For this reason, starting with a modified natural product such as taccalonolide E is attractive since nature has already done part of the work.

Nonetheless, we have come up with a new plan for the generation of a derivative with deoxygenation in both ring A and B. this approach makes use of chemistry that we have gained some experience with recently in the connection with a different project. The advantage is that all four oxygen atoms in these two rings will be deleted using the same chemistry. After acid-catalyzed ring opening of the epoxide and reduction of the ketone, all four hydroxyl groups will be activated with phenylthionochloroformate and then removed using tributyltinhydride as shown below. The advantage of this approach is that one can use a large excess of one reagent to push the derivatization of all of the oxygen atoms in ring A and B to completion. In contrast, different methodologies for the removal of individual atoms require excess of several different reagents with the entailing purification problems. While these can be solved on moderate scale between 20 to 50 mg where losses can be tolerated, on the low milligram scale this approach poses real problems.



Key Research Accomplishments

None this year

Reportable outcomes

Ms. Anokha S. Ratnayake who was partially supported by this award successfully defended her Ph.D. degree this summer and will officially graduate in December 2003.

Conclusions

This past year we have hit a major road block in that the preparation of analogs with multiple functional group deletions is more difficult than we had anticipated. As outlined above we have plans that may help us get around these obstacles but do not constitute major changes in the plan. New graduate students have not joined the group in the past year nor have we received applications for postdoctoral positions that are worth considering. Hence the lack of manpower remains a troubling issue for progress.

References

[1] Tinley, T.L; Randall-Hlubek, D. A.; Leal, R. M.; Jackson, E. M.; Cessac, J. W.; Quada, J. C. Jr.; Hemscheidt, T.K.; Mooberry, S. L. "Taccalonolide E and A: Plant-derived Steroids with Microtubule-Stabilizing Activity" Cancer Research **2003**, *63*, 2311-3220.